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Award Number: DAMD17-03-2-0016

TITLE: Development and Evaluation of New Products for the Far-Forward Care of Combat Casualties with Acute Lung Injury

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REPORT DATE: February 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2005	Annual (1 Feb 2004 - 31 Jan 2005)

4. TITLE AND SUBTITLE
Development and Evaluation of New Products for the Far-Forward Care of Combat Casualties with Acute Lung Injury

5. FUNDING NUMBERS
DAMD17-03-2-0016

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Objective: To characterize the acute respiratory distress syndrome (ARDS) caused by chlorine gas (Cl_2). **Background:** Toxic industrial chemicals (TICs) have recently been identified as potential terrorist weapons. Several TICs act primarily on the respiratory tract, but more work is needed to define the pathophysiology and treatment of these injuries. **Methods:** Anesthetized female sheep (n=35, 42.4 kg ± 5.4 SD) were ventilated with 300 L of a Cl_2/air/oxygen mixture over 30 min. Doses were: 0 ppm (Control, Group 1); 120 ppm (Low Dose, Group 2); 240-350 ppm (Medium, Group 3); and 400-500 ppm (High, Group 4). After injury they were maintained for 96 h in an animal ICU. Gentle mechanical ventilation (peak airway pressure < 40 cmH_2O) was required to limit barotrauma. Cardiopulmonary data were collected every 6 h, and CT scans daily. The multiple inert gas elimination technique (MIGET) was used to characterize the etiology of hypoxemia. **Results:** Lung function was well maintained in Group 1; Cl_2 caused immediate and sustained acute lung injury (PaO_2-to-FiO_2 ratio, PFR<300) in Group 2, and ARDS (PFR<200) in Groups 3-4 (ANOVA $p<.0001$ between/within groups). Cl_2 also rapidly caused hypotension and decreased cardiac output, lasting 48 h in survivors. All animals in Groups 1-2 survived 96 h. Kaplan-Meier analysis showed dose-related differences in survival (Log Rank test, $p<.0001$). Logistic regression identified 280 ppm as the lethal dose 50%. CT and histopathology demonstrated lesions of both small airways and alveoli. MIGET showed diversion of blood flow from normal to true-shunt lung segments. **Conclusions:** Cl_2 causes severe, dose-related lung injury, with features seen in both smoke inhalation (small airway lesions) and ARDS secondary to systemic disease (alveolar-endothelial lesions). This model will be used to test the Intravenous Membrane Oxygenator being developed by ALung Technologies, Inc. in collaboration with the University of Pittsburgh.

14. SUBJECT TERMS
Chlorine, acute respiratory distress syndrome, inhalation injury

15. NUMBER OF PAGES
27

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

This project represents a collaboration between the U.S. Army Institute of Surgical Research (ISR), Fort Sam Houston, TX, and the McGowan Institute of Regenerative Medicine at the University of Pittsburgh, Pittsburgh, PA. In addition, the intravenous membrane oxygenator (IMO) catheter developed at the University of Pittsburgh, is now being manufactured and refined by ALung Technologies, Inc., in collaboration with the University. The goal is to improve our ability to treat casualties with acute respiratory distress syndrome (ARDS), as well as to support Homeland Security by better understanding lung failure secondary to inhalation of a highly toxic, widely available industrial chemical. The objectives of this project are as follows:

Task 1. Evaluate the present IMO catheter in an ovine model of severe ARDS secondary to inhalation injury.

a. Develop and characterize an ovine model of ARDS secondary to inhalation of chlorine gas. Determine the lethal dose 50% (LD_{50}) at 96 hours for this model. Describe the pathophysiology of hypoxia in this model.

b. Evaluate the efficacy of the present IMO catheter in the LD_{50} inhalation injury model with respect to mortality at 96 hours, as well as various secondary endpoints.

Task 2. Refine and test an IMO catheter with an anticoagulant polymer coating on the surface of the hollow fiber membranes such that the requirement for systemic anticoagulation can be reduced or eliminated.

c. Develop a new polymer-coated IMO catheter(s). Characterize the gas-exchange performance of the new catheter(s) in vitro at the University of Pittsburgh Artificial Lung Lab.

d. Determine the level of anticoagulation needed, if any, for animals treated with the new polymer-coated catheter.

This report provides a summary of work conducted to date on the project (Tasks 1a and 2 objectives), by the ISR and by the University-ALung teams.

BODY

Part 1. Work Performed by the U.S. Army Institute of Surgical Research

Overview

Over the past year, we completed the animal work on the first phase of this project. Specifically, we conducted work to determine the lethal dose 50% (LD_{50}) for chlorine gas inhalation injury, and to characterize the injury from the standpoint of physiologic, histologic, radiographic, and biochemical changes. This report will focus on these first phase results.

In addition, we commenced an addendum specifically intended to assess the effects of chlorine gas inhalation injury on ventilation-perfusion (V/Q) matching in the injured lung by means of the Multiple Inert Gas Elimination Technique (MIGET). Finally, we have worked with the University of Pittsburgh and ALung Technologies, Inc. to refine the design of the Intravenous Membrane Oxygenator (IMO) catheter.

Regulatory compliance

This study was approved by the institutional Animal Care and Use Committee. The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other federal statutes and regulations relating to animals and studies involving animals, and by the 1996 *Guide for the Care and Use of Laboratory Animals* of the National Research Council. All animals were maintained in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Personnel

In support of this project, a round-the-clock Animal Intensive Care Unit (ICU) was operated at the ISR. Dr. Andriy Batchinsky, a General Surgeon and previously a National Research Council fellow at the ISR, returned from Ukraine in order to work full time on this project in Jan 2005 as an Associate Investigator. David Martini, M.S., a qualified veterinary technician, continued as the Laboratory Supervisor. Candace Baird, RVT, LAT and Denise Hardin, LAT (the latter replacing Michael Lucas) served as veterinary technicians on the project. The attending veterinarian was Dr. James Fudge (replacing Dr. Ronald Walton). Bryan Jordan, RN, CCRN, a critical care nurse from the Army Burn Center, continued to serve as Chief Nurse for the Animal ICU.

Surgery

Anesthetized female sheep (n=35, 42.4 kg ± 5.4 SD) (Josh Talley Ranch, Uvalde, TX), negative for *C. burnetti*, were used to complete this first phase of the project. (This number does not include additional animals used for training purposes.) Animals were fed up until the time of surgery, since post-injury feeding in these deeply sedated animals

was not feasible. Otherwise, management of these animals was as described in last year's report.

Computed tomography scanning

Computed tomography (CT) scans of the chest were performed in the animal imaging room immediately after surgery; at 2, 24, 48, 72, and 96 hours after injury or sham injury, as described in last year's report.

Intensive care

Animals were maintained postoperatively in the animal ICU, with 24-hour a day, one-on-one care by qualified technicians. After injury, all animals, injured and control, were maintained in deep sedation with midazolam and ketamine as described in last year's report. Buprenorphine (Buprenex), 0.3 mg/ml, 0.25-2 ml i.v., was given for pain.

Intravenous fluids were given according to the program described in last year's report. In addition, we learned that high-volume losses of highly alkaline saliva can cause a non-gap metabolic acidosis in this species. This became evident in a series of animals, both injured *and uninjured*, which were more deeply sedated after injury than their predecessors. A pathologic etiology for this problem, such as renal tubular acidosis, lactic acidosis, or ruminant acidosis, was ruled out. Furthermore, we found that magnitude of salivary losses and of the resulting metabolic acidosis can be decreased by elevation of the head and by maintaining a level of consciousness sufficient to allow swallowing. In addition, it was often necessary to replace salivary losses by giving additional i.v. fluid as sodium bicarbonate ($NaHCO_3$). In both injured and uninjured control animals, $NaHCO_3$, 150 mEQ per liter of sterile water, is now given in order to maintain an arterial base excess level of –5 to 5. The maximal rate of this infusion is 100 cc/h, which was determined to be the usual maximal rate required for replacement of salivary losses in uninjured animals.

Mechanical Ventilation

Animals were maintained on a Servo 300 mechanical ventilator (Siemens). The strategy for ventilator management described in last year's report was followed.

Physiologic measurements

Procedures for the collection of physiologic data are as described in last year's report.

Chlorine injury

Chlorine gas delivery methods were unchanged from last year's report. No safety-related problems with chlorine delivery or handling have occurred. Doses were:

0 ppm (Control, Group 1)

120 ppm (Low Dose, Group 2)

240-350 ppm (Medium, Group 3)

400-500 ppm (High, Group 4)

Necropsy

The necropsy procedures were unchanged from last year's report.

Multiple Inert Gas Elimination Technique

The Multiple Inert Gas Elimination Technique (MIGET) is the gold standard method for determining the etiology of hypoxia following injury, and quantifies the amount of shunt, ventilation-perfusion mismatching, diffusion limitation, and hypoventilation in the animal. MIGET was adopted for use in this project by Dr. Andriy Batchinsky of the ISR as described in last year's report, and was reintroduced into the study with his return from overseas in Jan 2005.

Results

The following table summarizes the outcome for those animals used to complete the first phase of this project:

ID number	include	dose, ppm	weight	live time	outcome	pneumothrx
002	0	120	33.8	56.25	0	1
023	0	120	35.5	54	0	1
201	0	120	38.2	83.5	0	1
024	0	120	34.1	95	1	0
044	0	120	45.0	57	0	1
042	0	120	40.0	68.75	0	0
045	0	240	41.1	39.5	0	0
AT-614	0	500	35.8	0	0	2
H-049	0	0	36.8	96	1	0
H-039	0	0	33.6	96	1	0
H-018	0	0	37.2	96	1	0
110	0	0	47.4	96	1	0
113	0	0	50.6	96	1	0
115	0	0	46	75	0	0
126	0	240	38	0	0	2
046	1	240	38.8	81.25	0	0
049	1	240	49.0	96	1	0
A-506	1	500	38.0	21.25	0	0
042-2	1	0	36.8	96	1	0
A-784	1	240	37.8	65	1	0
A-785	1	0	39.0	96	1	0
A-782	1	350	33.2	96	1	0
A-783	1	500	36.4	40.75	0	0
AT-610	1	500	34.6	10.75	0	1
H-035	1	400	33.2	8.25	0	1
H-005	1	0	33.4	96	1	0
T-612	1	400	44.4	96.25	1	0
H-091	1	400	39.6	25.9	0	0
H-033	1	400	34.4	12.25	0	0
H-088	1	400	44.6	22	0	0
108	1	400	46.2	9.2	0	0
106	1	400	50.8	25	0	0
109	1	400	43.6	96	1	0
107	1	400	43.2	30.5	0	0
112	1	0	48.6	96	1	0
111	1	300	47.2	25.1	0	0
114	1	300	43	13.15	0	0
124	1	0	43.4	96	1	0
125	1	0	46.6	96	1	0
123	1	240	44.8	96	1	0
400	1	240	47	29.3	0	0
139	1	240	43.6	50.1	0	0
401	1	240	53.2	96	1	0
402	1	240	48.6	96	1	0
326	1	120	40.4	96	1	0
1006	1	120	46.8	96	1	0
1009	1	120	44.6	96	1	0
10008(A00B)	1	120	41.3	96	1	0
1043	1	120	46.2	96	1	0
1042	1	120	44.4	96	1	0

Table 1: outcome data. ***Include****, was the animal included in the study, 1=yes, 0=no.* ***Dose, ppm****, dose of chlorine gas in parts per million.* ***Weight****, pre-injury weight in kg.* ***Live time****, survival time of animal in hours.* ***Outcome****, did the animal survive to the end of the 96 hour study, 1=yes, 0=no.* ***Pneumothrx****, did the animal develop a pneumothorax during the study, 1=yes, 0=no, 2=yes (during injury).*

Lung function was well maintained in Group 1; Cl_2 caused immediate and sustained acute lung injury (PaO_2-to-FiO_2 ratio, PFR<300) in Group 2, and ARDS (PFR<200) in Groups 3-4 (see Figure 1; ANOVA p<.0001 between/within groups).

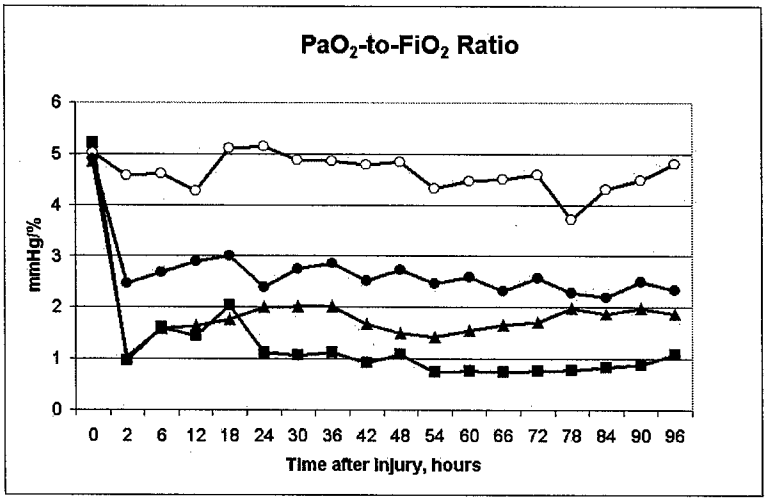


Figure 1: PaO_2-to-FiO_2 ratio as a function of time post-injury. ***Open circles****, group 1;* ***closed circles****, group 2;* ***triangles****, group 3;* ***squares****, group 4.*

Cl_2 also rapidly caused hypotension and decreased cardiac output, lasting 48 h in survivors.

All animals in Groups 1-2 survived 96 h. Kaplan-Meier analysis showed dose-related differences in survival (Log Rank test, p<.0001):

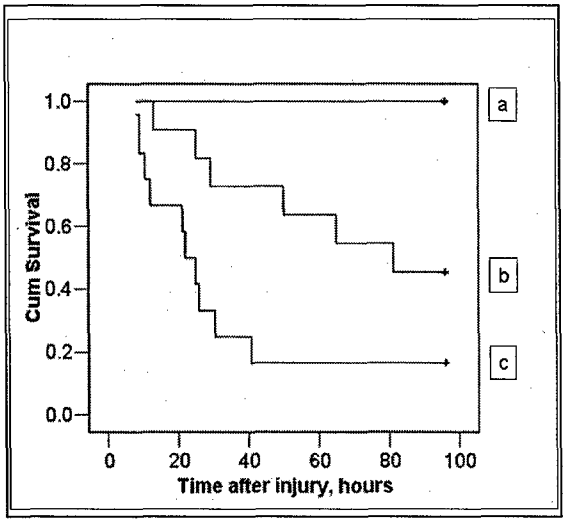


Figure 2: Kaplan-Meier survival curves. ***a****, groups 1-2;* ***b****, group 3;* ***c****, group 4. Log Rank test overall: p<.0001. Pairwise comparisons: a vs. b, p=.0032; a vs. c, p<.0001; b vs. c, p=.0291*

Logistic regression identified 280 ppm as the lethal dose 50%. In this analysis, probability of survival is given by the following equation:

$$P(\text{survival}) = e^k/(1+e^k), \text{ where } k = -0.014(\text{dose}) + 3.939.$$

The following graph gives the predicted survival (based on the above equation) as a function of chlorine dose:

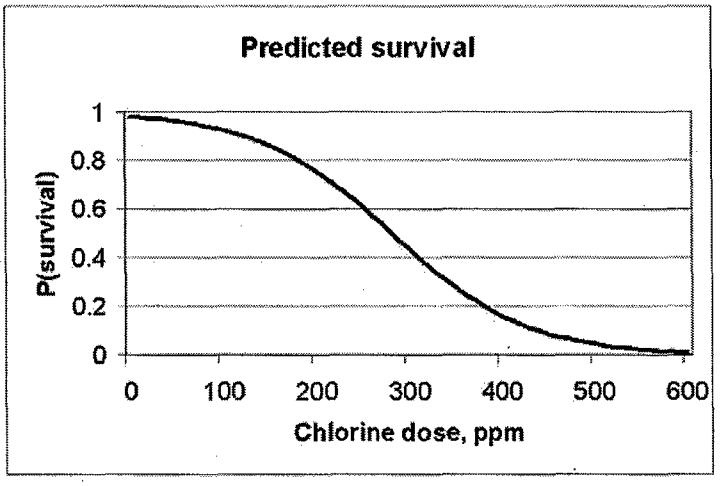


Figure 3: predicted survival as a function of chlorine dose. Visually, it is apparent that the LD_{50} *is 280 ppm.*

CT scans

We are currently performing off-line analysis of sheep CT scans using 3D Doctor software.

MIGET

MIGET work was suspended during Dr. Batchinsky's absence and has been resumed now that he has rejoined this project. These results will be reported in next year's report, but indicate an immediate, massive, and sustained increase in true shunt following injury, with an increase of lesser extent in blood flow to poorly ventilated lung segments.

Pathology

We continue to perform histopathology on all animals in this study. Results are similar to those reported last year. We intend to develop a scoring system for the histopathologic severity of injury, although the multifocal variability of this injury may make such scoring difficult.

Biochemistry

Biochemical indices of oxidative damage are being assayed by Dr. Michael Dubick at ISR, and results are pending.

Discussion

Our conclusions to date are similar to those initially reported in last year's report, and can be summarized as follows.

- Consistent with the findings of other authors, chlorine gas in the ppm range produces a highly lethal acute lung injury. With respect to survival, survival time, and oxygenation (Figures 1-3), the injury is clearly dose-responsive (worse with increasing dose).

- We have found that with modern methods of ventilatory management, the lethal dose fifty percent (LD_{50}) in our study at the four-day mark is 280 ppm. This is higher than that reported in the existing literature, which suggested that the one-day LD50 might be about 120 ppm. This may reflect differences in patient management and/or in inter-species tolerance to the injury. Gentle mechanical ventilation is important to reduce the incidence of pneumothorax.

- A characteristic bimodal declination in oxygenation was seen (Figure 1); with some post-injury improvement in PaO_2-to-FiO_2 ratio in most animals, followed by a later secondary decline.

- The immediate post-injury period was also associated with hypotension, relative bradycardia, and decreased cardiac output. These findings may prove troublesome during catheter insertion (the catheter may decrease cardiac preload). Thus, during the next phase of the study, we will be prepared to support cardiac function with fluids and pressors as needed.

- Both anatomically and functionally, chlorine gas inhalation injury appears to occupy a middle ground on a continuum between ARDS caused by systemic disease (such as sepsis) or direct pulmonary contusion on the one hand, and smoke inhalation injury on the other.

    - The former type of ARDS features alveolar injury and an increase in true shunt. By contrast, smoke inhalation injury features small airways injury and an increase in blood flow to poorly ventilated (partially obstructed) lung segments.
    - Chlorine clearly diffuses more readily into the alveoli than does wood smoke, thus causing both small airways and alveolar injury, and causing an increase in true shunt more so than an increase in blood flow to poorly ventilated lung segments.
    - Thus, e.g., therapies which function well in smoke injured patients may not work as well in chlorine injured patients. More work is needed to define better therapies for victims of this type of injury.

Part 2. Work Performed by ALung Technologies, Inc.

During this reporting period, the following activities were carried out by ALung Technologies, Inc. This company has joined this project as a subcontractor, and is carrying out further development and production of the catheter with scientific input from Dr. Brack Hattler and colleagues of the University of Pittsburgh Artificial Lung Laboratory.

1. **Completed the heparin-coating process to provide a stable coating during the 7-day wash test.** ALung has completed its development of a stable, plasma resistant and anti-thrombogenic coating. A heparin coating process has been developed which provides both persistent heparin activity and acceptable gas permeability. Utilizing a 2-step in-solution illumination and spray process, initial heparin activity levels of 13 mU/cm^2 have been achieved. After seven days of washing, heparin activity persists at a level of 8 mU/cm^2. Work is underway to validate the heparin coating process for 200 micron fibers.

2. **Redesigned the catheter to utilize a smaller 200 micron fiber that provides a higher surface area.** ALung has recently completed the work necessary to build intravenous membrane oxygenator (IMO) devices using smaller-diameter hollow-fiber membranes. By reducing the diameter of the hollow fibers, the surface area available for gas exchange can be increased, secondary to the increased surface-area-to-volume ratio of the smaller fiber. Decreasing the outer fiber diameter from 250 microns to 200 microns has allowed for up to 25% more surface area to be incorporated into a device of the same size (as measured by the size of the fiber bundle pots). Work is currently underway to evaluate the insertion compatibility of 200 micron devices. Insertion requirements may require that the number of fibers be reduced from the theoretical maximum, but some increase in surface area from the 250 micron fiber devices is ultimately expected.

3. **Prototyped a new proximal connector.** The proximal connector has been designed to be a mass-produced, one-piece, quick-connect device. It mates with the driveline in such a way that the gas lines cannot be connected incorrectly at either end of the driveline. The internal channels offer better transitions than previous designs, thus improving flow. The connector features an ergonomic design with a suture feature and strain relief for the shaft tube, that will provide a good outlet for venous-access-port connections.

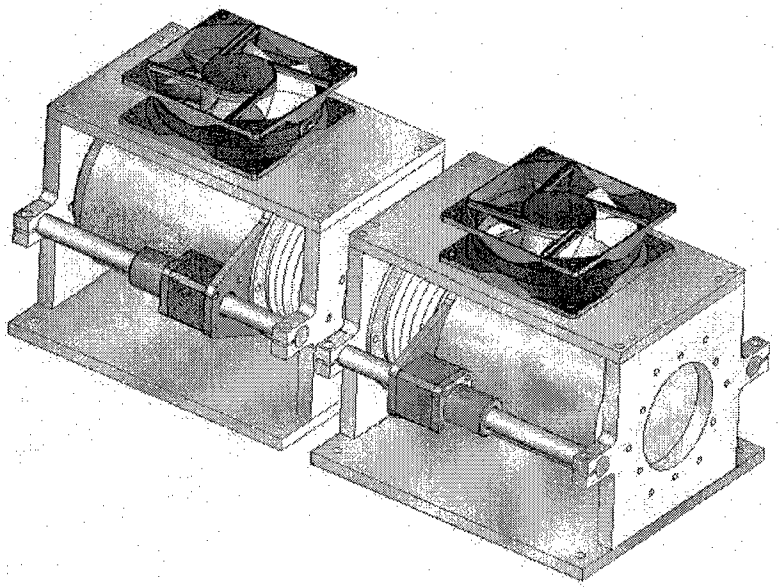
4. **Developed a multi-lumen design for the delivery of heparin, and drug delivery/sampling.** In collaboration with Avalon Laboratories, a manufacturer of dip-molded, coiled-reinforced cannulas, ALung has completed feasibility work and has produced a prototype multi-lumen shaft tube. In previous designs of the IMO, the shaft tube consisted of a single lumen and served only as a pneumatic pathway. In addition to serving as a pneumatic pathway, the new shaft tube will also provide for central venous access similar to a central venous catheter. Four additional lumens will be included into the wall of the new shaft tube. Three of these lumens will be for

drug/fluid administration, blood sampling, and pressure monitoring with flow rates similar to a 7 Fr triple lumen catheter (one 16 Ga. lumen and two 18 Ga. lumens). The fourth lumen will be for localized heparin administration. Heparin will be administered at a low flow rate through the distal lumen just below the fiber bundle to assist in anticoagulation. Work is currently underway to complete a final prototype by August 2005.

5. **Developed a voice coil console system that makes the system significantly more quiet, and allows the catheter to be driven up to 600 bpm.** Development and tests from previous console designs that utilized a pneumatic drive system led ALung to conclude that a more viable solution would be to use a "voice-coil" type approach, employing 2 opposing linear-drive motors with attached metal bellows to generate the required pressures and vacuum to pulsate the Hattler Catheter balloon to the higher frequencies that would yield improved gas exchange.

   The primary design objective was to pulsate the catheter balloon with complete inflation and deflation at frequency ranges between 60 to 600 bpm. Secondary design objectives were to pulse at 2 different frequencies simultaneously (a carrier frequency at 60 -180 bpm and a high frequency modulating pulse at 180 - 600 bpm), to make the system significantly more quiet, and to reduce current draw.

   The following (Figure 4) is a picture of the design:



   Results from a prototype unit have realized all of the design goals set forth above. Subsequently, several complete consoles are being constructed for the animal. and human trials.

6. **Completed development of the piggy-back insertion system.** The IMO is a large transcutaneous catheter that is to be inserted into the vena cava via the femoral vein. A percutaneous insertion method has been developed to permit the catheter to be placed in the emergency department (ED) or intensive care unit (ICU), or by an interventional radiologist. Incorporating the Seldinger Technique, the customary procedure for percutaneously inserting a device into a vessel, a thin-wall needle is inserted into the femoral vein. A guidewire is passed through the needle and into the vena cava, with a portion of the wire remaining outside the body. ALung Technologies has developed a unique and proprietary method for readily dilating the tissue and advancing a large introducer sheath with minimal blood loss. The sheath is a conduit which will permit placement of the catheter into the venous system. With the needle removed, a "guide dilator" is inserted over the wire and is advanced into the vena cava. A portion of the guide dilator also remains outside the body. A larger dilator with an integrated sheath is inserted over the guide dilator. This dilator increases the size of the insertion site and permits placement of the sheath in the femoral vein. With the guidewire and dilators removed, the catheter is inserted through the sheath. Once in place, the sheath is removed from the insertion site and is torn away from the device. This method has been successfully tested on cadavers and animal models. The dilators are radio-opaque, facilitating insertion under fluoroscopy if desired. However, the device can be also inserted without fluoroscopic visualization.

Figure 5 – Piggy-back Dilators

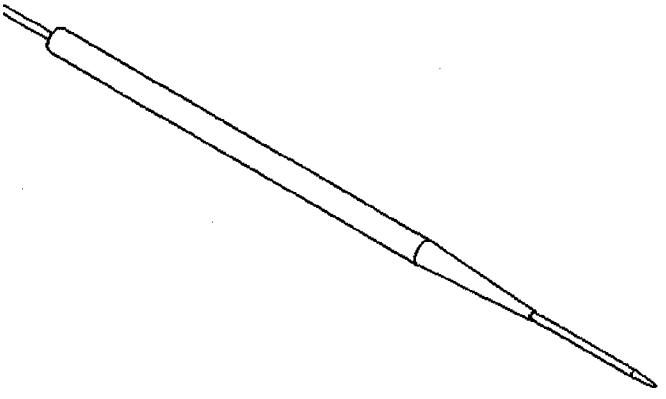
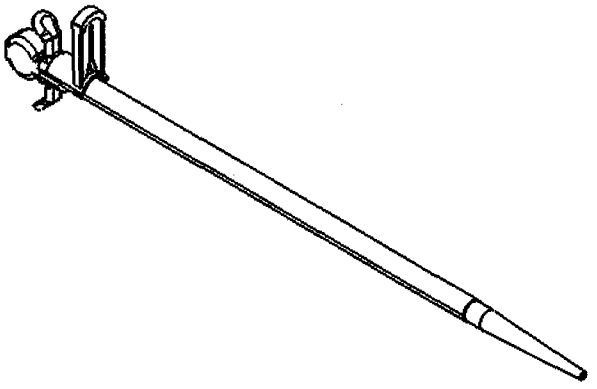


Figure 6 – Piggy-back Dilator with Tear-away Sheath



7. **Completed packaging for shipment, and return of used catheter for analysis.** A single shipping container has been designed to deliver the IMO System (i.e., catheter and all associated disposable accessories). This container will assure the devices are delivered in a sterile and functional condition. All elements required to insert, remove and return the device, as well as any disposables associated with the console, will be included in the container. Sterile devices will be individually packaged in pouches. The pouches will be subdivided into containers (boxes) within the shipping container. The removal and return accessories will be packaged separately within the container and will be clearly marked. Any disposables associated with the console will also be packaged separately within the container and will be clearly marked. This will allow these accessories to be maintained by the hospital until they are required. Once a catheter has been explanted, a kit for properly preparing and returning the device has been designed. The packaging assures the device will be returned in a sealed and undamaged condition.

8. **Refined catheter manufacturing process to improve yield and reduce failure rate; set up manufacturing and quality control testing in a clean room environment.** All pilot and normal production activities take place in a clean room at ALung Technologies, Inc. ALung designed and installed a Class 10,000 clean room (10,000 0.5 micron particles per cubic foot of atmosphere) in December 2003. The clean room is monitored and maintained as a Class 100,000 room (100,000 0.5 micron particles per cubic foot of atmosphere), although limits are normally within that of a Class 10,000 clean room. ALung periodically performs particulate monitoring, viable particulate monitoring and cleaning according to an approved schedule.

   Currently, Alung is in the process of establishing a batch-release-contract ethylene oxide sterilization process. The batch-release program has been designed using current ISO and FDA guidance documents in which all disposables will be evaluated for sterility, bioburden, endotoxins, and ethylene oxide residual. The batch release is a good choice for Alung due to the likely potential for device-design changes. A

validated sterilization process is a design-control deliverable prior to design release to full manufacturing.

ALung has been in the process of moving from a research and development and prototyping operation to a GMP facility manufacturing a class III medical device and accessories. To date we have established incoming and in-process quality-control inspections, as well as approved and validated work instructions to build and package the device and accessories in the clean room. These work instructions identify manufacturing processes, specific QC steps and final QA acceptance prior to sterilization. Critical process and testing parameters are measured and captured on routers, which in turn allows ALung to monitor and thus continuously improve our production processes.

Part 3. Work Performed by the University of Pittsburgh

During this reporting period, the following activities were performed by the McGowan Institute of Regenerative Medicine at the University of Pittsburgh, under the direction of Dr. Brack Hattler.

1. **Completed training on Daedalus drive console operation.** Heide Eash and Catherine Roukous worked with Bob Romano of ALung Technologies, Inc. to learn operation and troubleshooting of the Daedalus drive console designed by ALung. The console drives the balloon pulsation and runs the sweep-gas pathway of the device. This training included the CO_2 analyzer, which is a different version than used currently in the University of Pittsburgh laboratory. Calibration procedures and troubleshooting for this instrument were examined. Possible issues related to shipping were analyzed to enable Ms. Eash and Ms. Roukous to troubleshoot the equipment once it arrived in San Antonio. After this training, a revised manual was written to include procedural clarifications and additional troubleshooting.

2. **Finalized protocols for device insertion, operation, and removal.** Heide Eash worked with Vince Testa of ALung to learn the improved method of insertion and removal of the catheter which was developed by ALung. She worked with Bob Romano regarding operation of the catheter (i.e. using the Daedalus console). Protocols were updated and finalized after presenting these methods during the "Demo Training" in November in San Antonio. Suggestions of Dr. Cancio and his team as well as Dr. Hattler were incorporated into the protocol to make the insertion, operation, and removal of the catheter as user-friendly as possible.

3. **Finalized data sheets for all experimental data collection.** All data collection sheets (case report forms) regarding the catheter were finalized for use in the experimental procedures. These forms were then presented to the San Antonio group for review. These forms include:

    a. **Information Datasheet** which records crucial experiment information including the implantation start time and lines placed, equipment serial numbers and zeros, baseline measurements, animal information (weight, sex, breed), device information (serial number, balloon size, etc), and personnel present.

    b. **Gas Exchange Datasheet** for the collection of beat rate, duty cycle, chamber pressure readings, sweep gas flow rate, $\%CO_2$ exiting the catheter, Catheter pressure drop, and femoral vein pressure (pCO_2/pO_2).

    c. **Cardiac Output Measurements** to record all three trials for each cardiac output measurement and the average of these.

d. **Critical Measurements sheet** including a drawing of where the catheter was located within the animal and vessel lengths and diameters.

e. **Critical Measurements and Necropsy** including pre-necropsy measurements of the jugular vein and vessel lengths during necropsy. Also included is anatomy presentation and catheter examination upon exit.

f. **Hematological Data sheet** for the collection of Femoral Vein, CVP, and Arterial pCO_2 and pO_2 measurements, as well as hematocrit and ACT levels.

g. **Hemodynamic Datasheet** for recording of MAP, CVP, and Femoral Vein pressures as well as Cardiac Output measurements.

h. **ICU Collection Matrix** stating when measurements should be taken throughout the experiment (including $\%CO_2$ exhaust, pressures, ACT, and cardiac output).

i. **Special Notes** for the recording of any information gathered during the experiment that is not included within other sheets (e.g. problems at insertion, animal unstable, etc).

4. **Initial selective perfuser experiments of the catheter, and flow visualization.** Four quarter regions around the fiber bundle were selectively perfused to look for non-uniformities in gas exchange within these different regions in an effort to improve gas exchange capabilities. Also, flow visualization tests were conducted, to compare the gas exchange data with actual fluid flow velocities and fiber bundle velocities (i.e. fiber bundle movement with pulsation). Selective perfusion studies were performed in a mock vena cava flow loop with 3 LPM flow, using helium as the sweep gas (to eliminate uneven flow distributions with the perfusion setup). A preliminary 15-45% variation between the different regions was seen for carbon dioxide exchange. Flow visualization was performed using Particle Image Velocimetry (PIV) at a 120 BPM balloon pulsation rate to determine the fluid velocity vector field around the catheter. Top and bottom quarter regions showed a variation up to 5 cm/s and 0 cm/s for the right and left quarters. Further investigations of both selective perfusion and PIV are being undertaken to further understand the catheter performance.

5. **Random balloon pulsation of the catheter.** In an effort to further improve the gas exchange performance of the catheter and to disrupt fluid entrainment within the fiber bundle, the balloon was randomly pulsated. Two different methods were undertaken; these were random pulsation rates and random volume delivery to the balloon. A mock vena cava test loop was utilized to characterize gas exchange. Random pulsation did not significantly improve gas exchange compared to constant pulsation. Randomly delivered balloon volume also did not increase gas

exchange when compared to constantly delivered balloon volume. Therefore, random pulsation was not investigated further.

KEY RESEARCH ACCOMPLISHMENTS

Chlorine Inhalation Injury (ISR):

- **Development of a dose-responsive model of chlorine-gas inhalation injury.** Inhalation injury secondary to chlorine gas (Cl_2) was shown to be dose-responsive, both respect to mortality, and with respect to oxygenation (PaO_2-to FiO_2 ratio). The lethal dose 50% (LD_{50}) was determined to be 280 ppm x 300 L under the conditions of this model.

- **Definition of a model of ARDS intermediate between systemic ARDS and smoke inhalation injury.** Computed tomography (CT) scanning and histopathology demonstrated lesions of both small airways and alveoli. The Multiple Inert Gas Elimination Technique (MIGET) showed diversion of blood flow from normal to true-shunt lung segments, with some redistribution to poorly ventilated segments as well. Thus, Cl_2--both anatomically and physiologically--causes an injury with features seen in both smoke inhalation (small airway lesions, increased blood flow to poorly ventilated segments) and ARDS secondary to systemic disease or pulmonary contusion (alveolar-endothelial lesions, increase in true shunt). These findings enhance our understanding of Cl_2 injury, and of ARDS generally.

Catheter Development (ALung Technologies, Inc. and University of Pittsburgh):

- **Development of a heparin-coating process to provide a stable coating.** A stable, plasma-resistant and anti-thrombogenic coating for the catheter was developed. A heparin coating process has been developed which provides both persistent heparin activity and acceptable gas permeability. Utilizing a 2-step in-solution illumination and spray process, initial heparin activity levels of 13 mU/cm^2 have been achieved. After seven days of washing, heparin activity persists at a level of 8 mU/cm^2.

- **Development of an improved method to drive the pulsation of the IMO balloon.** A "voice-coil" approach was developed, employing 2 opposing linear-drive motors with attached metal bellows to generate the required pressures and vacuum to pulsate the Hattler Catheter balloon to the higher frequencies that would yield improved gas exchange.

- **Development of a novel catheter insertion system.** A unique method for readily dilating the tissue and advancing a large introducer sheath with minimal blood loss was developed. The sheath is a conduit which will permit placement of the catheter into the venous system. With the needle removed, a "guide dilator" is inserted over the wire and is advanced into the vena cava. A portion of the guide dilator also remains outside the body. A larger dilator with an integrated sheath is inserted over the guide dilator. This dilator increases the size

of the insertion site and permits placement of the sheath in the femoral vein. With the guidewire and dilators removed, the catheter is inserted through the sheath.

- **Assessment of random balloon pulsation.** Random pulsation did not significantly improve gas exchange compared to constant pulsation. Randomly delivered balloon volume also did not increase gas exchange when compared to constantly delivered balloon volume.

REPORTABLE OUTCOMES

Cancio LC, Batchinsky AI, Martini DK, Jordan BS, Dick EJ, Fudge J, Baird CA, Lucas M, Hardin DE. Acute respiratory distress syndrome secondary to inhalation of chlorine gas. Submitted to the American Association for the Surgery of Trauma 2005 Annual Meeting, 22-24 Sep 2005, Atlanta, GA (awaiting notification).

Dick EJ Jr., Martini D, Jordan B, Walton R, Lucas M, Batchinsky A, Cancio L. An ovine model of acute respiratory distress syndrome (ARDS) secondary to inhalation of chlorine gas. Veterinary Pathology, 2004;41(5):588. Presented at the meeting of the American College of Veterinary Pathologists, Orlando, FL, Nov 2004.

CONCLUSIONS

Chlorine gas inhalation injury has been demonstrated to cause rapid and severe lung injury in the animal model. As it is a widely available toxic industrial chemical, we remain concerned that this gas, as well as other gases, could be used as weapons of opportunity by terrorists. During the remainder of this project, we will test the new intravenous membrane oxygenator (IMO) catheter as treatment for this injury.

The potential utility of the IMO catheter extends well beyond this type of lung injury, however. Far more common is ARDS secondary to sepsis, and due to blunt trauma and hemorrhagic shock. Furthermore, medical patients with acute exacerbation of chronic obstructive pulmonary disease (COPD) could be benefited from this type of technology, particularly if its use permits the avoidance, altogether, of endotracheal intubation and mechanical ventilation.

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